Isolation of bovine and human milk-derived exosomes: optimization of methods

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Introduction

- Released by intact mammary cells, exosomes are extracellular microvesicles (EMs) (30-100 nm diameter)[1] by which a mother may communicate important signals for the infant's developing gastrointestinal epithelial cells[2]. Exosomes can potentially be used to prevent and treat various neonatal gastroenterology disorders, but have yet to be fully understood.
- Due to the lack of optimized protocols for, and difficulty of, isolating exosomes from other constituents present in breast and bovine milk[3,4], research performed on exosomes may not be selective for these vesicles.
- Aim: to compare purification protocols for optimized yield and quality of exosomes. Both human and bovine milk were analyzed for optimal exosome isolation.

Methodology

Isolation:

- Exosome populations were derived from either a sample of bovine milk (whole milk, Sheldon Creek Dairy) or human breast milk (frozen at -80°C after extraction).
- All steps were performed in DEPC-treated water.
- The samples were prepared with the protocol in **Figure 1**.

Detection:

- Nanoparticle tracking analysis (Nanosight): detect the presence & concentration of vesicles based on size.
- Western blot: detect commonly found tetraspanins in exosomes, such as CD63 and CD81 receptors[1].

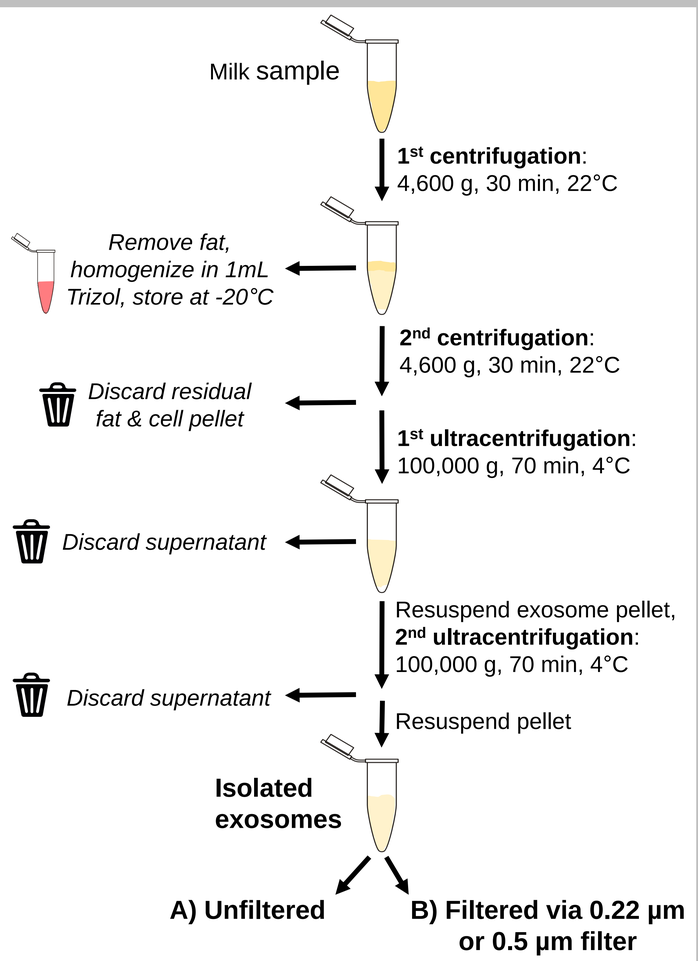

Figure 1. Protocol for exosome isolation

Results

A) Unfiltered – Breast Milk

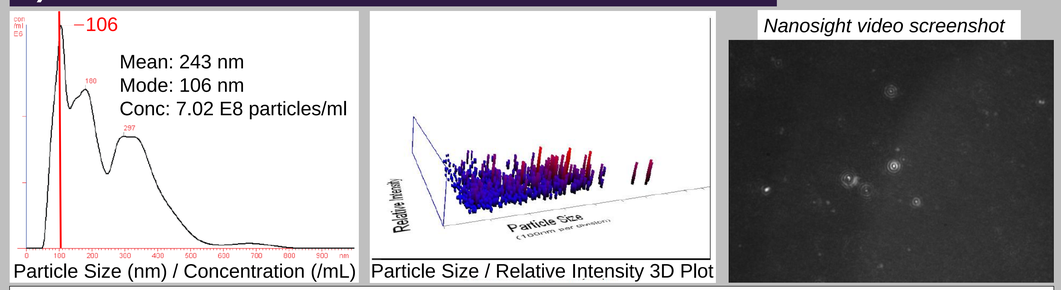

Figure 2. Isolated breast milk exosomes (1/10 dilution), unfiltered

B) Filtered (0.22 µm filter) – Breast Milk

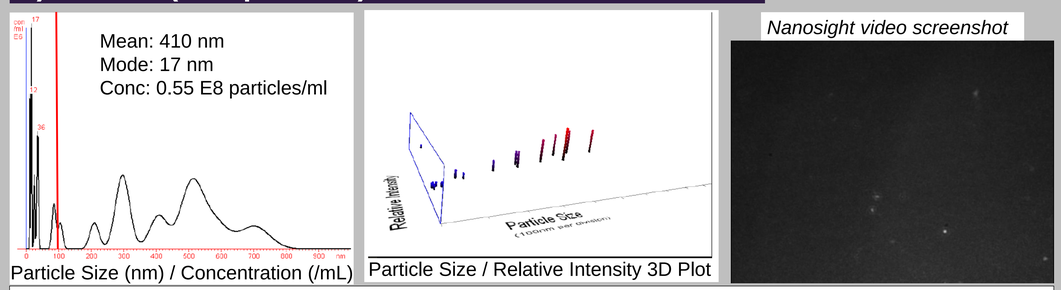

Figure 3. Isolated breast milk exosomes, filtered 1x with a 0.22 µm filter

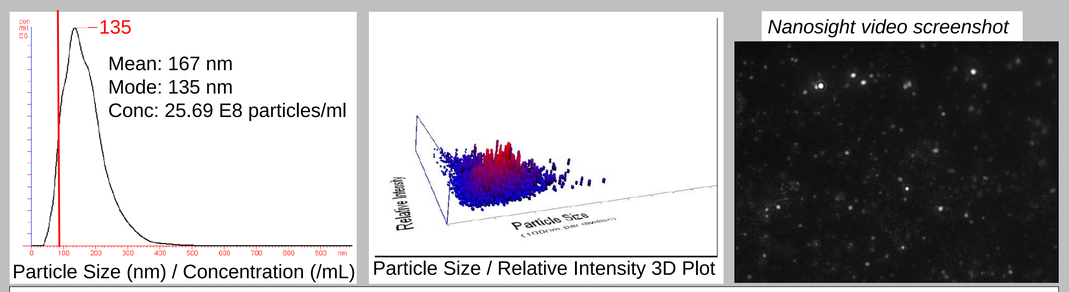

Figure 4. Isolated breast milk exosomes, filtered 1x with a 0.22 µm filter and 3x with a fresh 0.22 µm filter.

B) Filtered (0.5 µm filter) – Bovine Milk

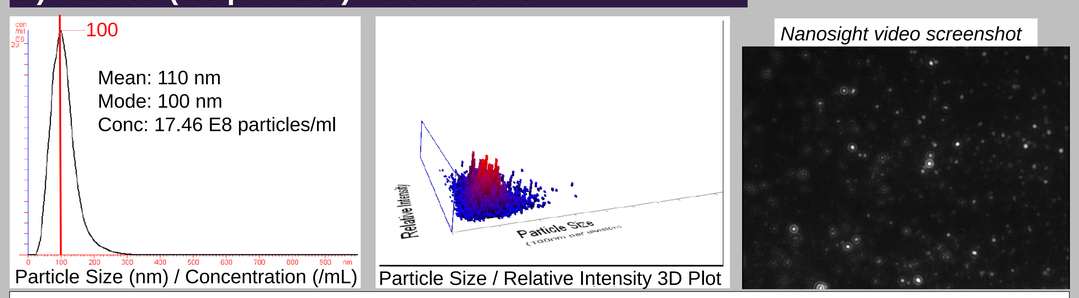

Figure 5. Isolated bovine milk exosomes, filtered 2x with two fresh 0.5 µm filters (1/320 dilution)

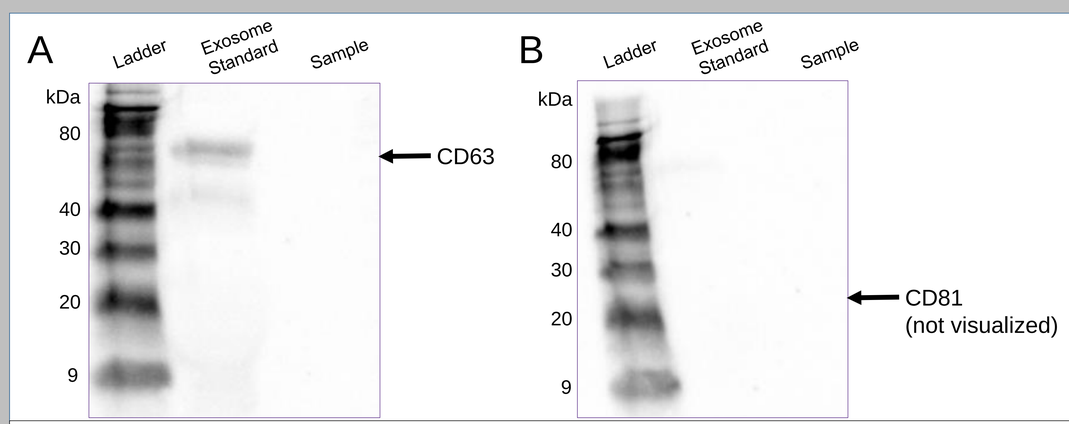

Figure 4. Western blot of bovine milk-derived exosomes. (A) Immunoblot to detect CD63 membrane protein. (B) Immunoblot to detect CD81 membrane protein.

Discussion & Conclusion

- The protocol used in this study demonstrated the ability to isolate exosome-sized EMs.
- Filtration using a 0.22 µm filter (**Figure 4**) or 0.5 µm filter (**Figure 5**) removed more debris and more exosome-sized EMs. Washing and diluting the sample enabled clearer imaging.
- Vesicles isolated could not be verified to contain exosomes via Western blot, suggesting presence of other EMs, or low concentrations of exosomes in the sample.
- Further optimization of exosome isolation will help to better study the characteristics of these EMs, including their potentially immunomodulating miRNA and protein contents.

Future directions:

- Performing the protocol in PBS to maintain membrane integrity better than DEPC-treated water, increasing the concentration of exosomes.
- Comparing results to sucrose density gradient centrifugation, which has been used to isolate exosomes[4].
- Further investigation of the capability of filtration for exosome purification.
- Use of flow cytometry and electron microscopy to obtain more precise evidence of the presence of exosomes[1].

References:

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